

IN THE ABSTRACT:

Please add the following Abstract page:

*amp
N/E*
A process is described for the production of ergosterol and its intermediate products,
using recombinant yeast and plasmids for transformation of yeast.

IN THE CLAIMS:

✓ Please cancel claims 1-26 without prejudice or disclaimer.

Please add the following new claims:

c3 Sub 27
~~27. A method of producing ergosterol or one or more intermediate products of its
biosynthesis, comprising,~~

~~a) designing a plasmid, into which the following genes are inserted:~~

- ~~i) **t-HMG**, an HMG-Co-A-reductase gene,
ERG9, a squalene synthetase gene,
SAT1, an Acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,~~

~~or~~

- ~~ii) **t-HMG**, an HMG-Co-A-reductase gene, and
ERG9, a squalene synthetase gene,~~

~~or~~

- ~~iii) **t-HMG**, an HMG-Co-A-reductase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,~~

~~or~~

CB
cont'd

- iv) ~~t-HMG~~, an HMG-Co-A-reductase gene, and
~~ERG1~~, a squalene epoxidase gene,
- or
- v) ~~ERG9~~, a squalene synthetase gene, and
~~SAT1~~, an acyl-CoA: sterol-acyl transferase gene,
- or
- vi) ~~ERG9~~, a squalene synthetase gene, and
~~ERG1~~, a squalene epoxidase gene,
- or
- vii) ~~SAT1~~, an acyl-CoA: sterol-acyl transferase gene, and
~~ERG1~~, a squalene epoxidase gene,
- or
- viii) one of the genes that is mentioned in i),
- b) transforming a microorganism with a plasmid mentioned in i) to vii), or,
simultaneously or in succession, with two or more of the plasmids mentioned in
viii), and
- c) culturing the transformed microorganism under conditions in which it produces
ergosterol.

Sub
cont

28. A method according to claim 27, wherein **ERG1**, a squalene epoxidase gene, is
further inserted into the plasmid mentioned in ii), iii) or v); or **SAT1**, an acyl-CoA: sterol-acyl
transferase gene, is further inserted into the plasmid mentioned in ii).

C3
and 2

29. The method according to claim 27, wherein the genes in each case with the plasmids are first introduced independently of one another into microorganisms of the same species.

30. The method according to claim 27, wherein the intermediate product is squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol, or ergosta-5,7-dienol.

31. The method according to claim 27, wherein the intermediate product is a sterol with a 5,7-diene structure.

32. The method according to claim 27, wherein the plasmid is YEpH2, YDpUHK3 or pADL-SAT1.

33. The method according to claim 27, wherein the microorganism is a yeast.

34 The method according to claim 33, wherein the yeast is the species *S. cerevisiae*.

35. The method according to claim 33, wherein the yeast is the strain *S. cerevisiae* AH22.

Sub D3

36. A yeast strain *S. cerevisiae* AH22 that contains one or more of the genes that are mentioned in i) of claim 27.

C3
Conf'd

37. The plasmid YEpH2, which comprises an **ADH**-promoter, a **t-HMG** gene, and a **TRP**-terminator, as shown in Fig. 1.

Sub
13

38. The plasmid YDpUHK3, which comprises an **ADH**-promoter, a **t-HMG** gene, a **TRP**-terminator, a gene for kanamycin resistance and a **ura3** gene, as shown in Fig. 2.

39. The plasmid pADL-SAT1, which comprises a **SAT1** gene and the **LEU2** gene of YEp13.

40. A method for producing ergosterol, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces ergosterol.

41. A method for producing an intermediate product in the biosynthesis of ergosterol, which is squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol, or ergosta-5,7-dienol, or a combination thereof, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces said intermediate product.

Sub-D4

42. The method according to claim 41, wherein the intermediate product is a sterol with a 5,7-diene structure.

C3
Cont'd

See
D4
Cont'd

43. An expression cassette that comprises a **t-HMG** gene flanked by an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene flanked by an **ADH**-promoter and a **TRP**-terminator.

44. An expression cassette that comprises a **t-HMG** gene flanked by an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene flanked by an **ADH**-promoter and a **TRP**-terminator, and an **ERG9**-gene flanked by an **ADH**-promoter and a **TRP**-terminator.

45. A combination of expression cassettes, which comprises

a) a first expression cassette, on which an **ADH**-promoter, a **t-HMG**-gene, and a **TRP**-terminator are located,

b) a second expression cassette, on which an **ADH**-promoter, a **SAT1**-gene and a **TRP**-terminator are located,

and

c) a third expression cassette, on which an **ADP**-promoter, an **ERG9**-gene and a **TRP**-terminator are located.

46. A method of producing a microorganism that can be used for producing ergosterol, comprising transforming a microorganism with an expression cassette according to claim 43.

47. The method according to claim 46, wherein the microorganism is a yeast.

C3
Cont'd

48. A microorganism which comprises an expression cassette according to claim 43.

49. The microorganism according to claim 48, which is a yeast.

50. A method for producing ergosterol, comprising culturing a microorganism according to claim 48 under conditions in which it produces ergosterol.

51. A method for producing one or more intermediate products in the biosynthesis of ergosterol, comprising culturing a microorganism according to claim 48 under conditions in which it produces said intermediate products.

52. The method according to claim 27, further comprising,

- d) after the culturing is complete, extracting the ergosterol and its intermediate products from the cells and analyzing them, and
- e) purifying the thus obtained ergosterol and its intermediate products, using column chromatography.

End
D3

53. A method for producing ergosterol or one or more intermediate products of its biosynthesis, comprising expressing in a microorganism a plasmid which comprises the following genes:

- i) t-HMG, an HMG-Co-A-reductase gene,
ERG9, a squalene synthetase gene,

C3
Cont'd

SAT1, an Acyl-CoA: sterol-acyl transferase gene, and

ERG1, a squalene epoxidase gene,

or

ii) **t-HMG**, an HMG-Co-A-reductase gene, and

ERG9, a squalene synthetase gene,

or

iii) **t-HMG**, an HMG-Co-A-reductase gene, and

SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

iv) **t-HMG**, an HMG-Co-A-reductase gene, and

ERG1, a squalene epoxidase gene,

or

v) **ERG9**, a squalene synthetase gene, and

SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

vi) **ERG9**, a squalene synthetase gene, and

ERG1, a squalene epoxidase gene,

or

vii) **SAT1**, an acyl-CoA: sterol-acyl transferase gene, and

ERG1, a squalene epoxidase gene,

or

viii) one of the genes that is mentioned in i).

due
to
cont